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# Study on the Carcinogenecity of N-Methyl-N'-Nitro-N-Nitrosoguanidine on Rat Stomach

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*Gastric tube administration of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) aqueous solution to Long Evans rats at a concentration of 1.5 mg/ml every day and 1 ml of saturated NaCl solution once a week for 15 weeks (wks) resulted in a high incidence of papilloma and squamous cell carcinoma formation in the forestomach. No other tumor or dysplastic change was found in other viscera except the stomach at the end of the experiment (36 wks).*

*Thirty-one rats died before 30 wks and only 5 rats survived until the completion of the experiment. The overall carcinoma induction rate and tumor induction rate were 29.3% and 60.0%, respectively. But if attention is focused on the 10 rats which survived more than 30 wks, 8 developed carcinoma and the other 2 developed only papilloma; therefore, the carcinoma and tumor induction rate would be 80% and 100%, respectively. No tumor was found in the control group of 10 rats.*

*Key words: carcinogen, MNNG, rat stomach, papilloma and epidermoid carcinoma.*

Gastric carcinoma is one of the most common malignancies found in many parts of the world. In order to study the pathogenesis, experimental production of gastric tumor has been going on for more than 50 years, but rarely has succeeded. The earliest attempt to induce gastric cancer was carried out by Fibiger in 1913[1], who erroneously concluded that spiroptera can form papilloma and squamous cell carcinoma in rat forestomach. In 1952, Hare et al[2] performed intramural injection of 20-methylcholanthrene (20-MCA) which induced adenocarcinoma adenoacanthoma and sarcoma on the glandular stomach of rats. In 1958, Stewart et al[3] also induced adenocarcinoma on the glandular stomach of mice by intramural injection of 20-MCA. In 1961, Stewart et al[4] found that by adding N,N'-2,7-fluorenylenebisacetamide (2,7-FAA) in the diet caused adenocarcinoma in the glandular stomach of rats, but the combination of these compounds was not more carcinogenic[5]. In 1963, Schoental[6] found that N-nitroso-N-alkyl-urethane could also cause adenocarcinoma in the glandular stomach of rats and mice. In 1969, Mori et al[7] by the instillation of 4-hydroxyaminoquinoline 1-

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oxide HCl, also induced adenocarcinoma on the glandular stomach. But in all of the above mentioned compounds, the tumor induction rate was very low. The carcinogenic activity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was first discovered by Mandell and Greenberg[8] in 1960. In the following years, its strong carcinogenecity, especially in production of gastric carcinoma, has been proven by many authors (Schoental[9] and Sugimura et al[10] in 1966). Because of the simplicity and high efficiency in production of gastric carcinoma, MNNG has been proven to be a new tool for studying experimental gastric carcinoma in animals. The present paper reports our experience in the production of gastric carcinoma in rats by MNNG.

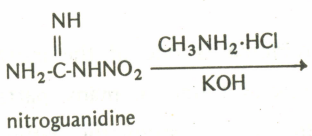
## MATERIALS AND METHODS

### Animals

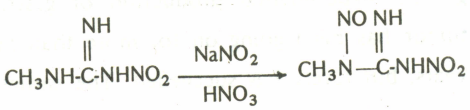
Fifty-one, 5-week-old male rats of the Long Evans strain, with average body weight about 80 gm at the beginning of the experiment, were housed 5 to a cage. The 51 experimental rats were divided into 2 groups. One group consisting of 41 rats were given MNNG, while another 10 were used for control. They were fed a basal diet for 12 wks. After then the experimental group was given MNNG in 1.5 mg/ml aqueous solution 1 ml every day and 1 ml of saturated NaCl solution once a week (both by intragastric tube) for 15 wks in addition to the basal diet. After the 15 wks of MNNG and NaCl administration, only the basal diet and water were given till the end of the experiment. The control group were only given a basal diet and tap water ad libidum throughout the experiment. The body weights were recorded every week. Rats that died during the experiment or that were killed after the completion of the experiment were autopsied. The stomach and other organs were grossly examined for tumor or other change and then fixed in a 10% formalin solution. Step serial section of the stomach and representative sections of all of the visceral organs were taken for histological examination.

### Chemical

The synthesis[11] of MNNG was accomplished according to the Scheme. Methylation of nitroguanidine with methylamine hydrochloride in aqueous alkaline solution gave methylnitroguanidine, which was reacted with aqueous sodium nitrite in acidic solution to give the product MNNG.




nitroguanidine




methylnitroguanidine MNNG

The pure MNNG, which is a yellow needle crystal and is slightly soluble in water, melts at 117-118°C (dec., from methanol). In our experiments, we suspended MNNG in deionized water at a concentration of 1.5 mg/ml and stored it in a dark bottle in a cold place to minimize the degradation by light. A fresh solution was prepared every 3 days.

## RESULTS

1. **Effect of MNNG Administration on the Growth Curve:**

The average body weight of rats in the MNNG group increased at the same rate as the control group for the first 8 wks after MNNG administration. After that period, the growth rate of the experimental group was a little more retarded than that of the control group. (Chart 1)

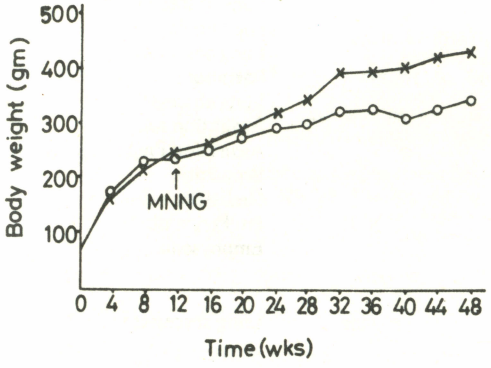



Chart 1. Effect of MNNG administration on the increase of the average body weight of rats. o——— o, experimental group (MNNG 1.5 mg/ml, 15 wks); x——— x, control group.

2. **The Death Rate of Rats:**

Thirty-one rats in the experimental group died before 30 wks of MNNG administration, and only 5 rats survived until the completion of experiment. Most of the animals that died during the experiment were found to have respiratory infections, including necrotizing purulent bronchitis, bronchopneumonia, lung abscess or purulent pleurisy or even pleuropericarditis. Many of them were apparently caused by aspiration pneumonia. (Table 1)

Table 1. Death Rate

| Duration (wk) | Control Group | Test Group |
|---|---|---|
| 1–10 | 0 | 5 |
| 11–20 | 0 | 18 |
| 21–30 | 0 | 8 |
| 30–36 | 0 | 5 |
| No. of died rats | 0 | 36 |
| Survival No. of rats | 10 | 5 |
| Total No. of rats | 10 | 41 |

3. **Tumor Formation:**

The tumors all developed on the forestomach, where it naturally covered the stratified squamous epithelium, which contained all squamous cell varieties — papilloma and squamous cell carcinoma. The only exception was one rat in which the carcinoma cells contained large clear cytoplasm, probably of sebaceous cell variety. The papillomas were often mutiple and small in size from just perceptible by naked eyes to 4 mm in diameter. They may form sharp conical protrusions onto the mucosa or sessile verrucous growths. The carcinoma varies from less than 1 mm in diameter to 4.0 x 1.5 x 1.0 cm in dimensions. Some carcinomas have already infiltrated into the serosa. But none was found to have metastasized. Most of the carcinomas were surrounded by multiple papillomas or by a zone of hyperkeratosis. The size of the tumor (either papilloma or epidermoid carcinoma) was roughly parallel to the duration of experiment and the carcinoma began to develop at 169 days after MNNG administration. (Table 2)

## DISCUSSION

The MNNG has been proven to be a

**Table 2. Tumor Formation of the Experimental Group which Received MNNG at 1.5 mg/ml for 15 wks**

| Rat No. | Experimental Period (Days) | Type of Tumor | Size of Tumor (mm) | Depth of Tumor | Remark |
|---|---|---|---|---|---|
| 1 | 21 D | | | | |
| 2 | 25 D | | | | |
| 3 | 25 D | | | | Lung abscesses |
| 4 | 38 D | | | | Bronchitis |
| 5 | 67 D | papilloma | 1x1x1 | | Aspiration Pn. |
| 6 | 72 D | papilloma | 1x1x1 | | Aspiration Pn. |
| 7 | 80 D | | | | Lung abscesses |
| 8 | 84 D | | | | Pneumonia |
| 9 | 87 D | | | | Pneumonia |
| 10 | 87 D | papilloma | 1x15 | | Pneumonia |
| 11 | 89 D | papilloma | multiple | | Pneumonia |
| 12 | 98 D | | | | Pneumonia |
| 13 | 101 D | papilloma | 1x1x1 | | Lung abscesses |
| 14 | 101 D | | | | Lung abscesses |
| 15 | 103 D | | | | Pneumonia |
| 16 | 105 D | | | | Lung abscesses |
| 17 | 112 D | papilloma | 1x1x1 | | Pneumonia |
| 18 | 113 D | papilloma | 2x2x1 | | Lung abscesses |
| 19 | 116 D | papilloma | 1.5x1x1 | | Aspiration Pn. |
| 20 | 122 D | | | | Aspiration Pn. |
| 21 | 123 D | papilloma | 4x3x1 | | Bronchitis |
| 22 | 126 D | | | | Mediastinal abscesses |
| 23 | 133 D | | | | Pn. Pericarditis |
| 24 | 154 D | papilloma | 3x3x2 | | Emphysema |
| 25 | 169 D | papilloma | 2x2x1 | | |
| | | epi. carcinoma | 5x5x5 | muscle | Lung abscesses |
| 26 | 170 D | papilloma | multiple | | |
| | | epi. carcinoma | 4x3x2 | mucosa | Pneumonia |
| 27 | 173 D | papilloma | 1x1x1 | | |
| 28 | 185 D | papilloma | multiple | | |
| | | epi. carcinoma | 1x1x0.5 | | |
| 29 | 197 D | | | | Marked autolysis |
| 30 | 197 D | | | | Marked autolysis |
| 31 | 210 D | papilloma | 2x2x1 | | |
| | | epi. carcinoma | 9x6x5 | submucosa | |
| 32 | 228 D | epi. carcinoma | 5x5x3 | submucosa | |
| 33 | 230 D | papilloma | 1x1x1 | | Lung abscesses Pericarditis |
| 34 | 231 D | epi. carcinoma | 20x10x3 | submucosa | Lung abscesses Pericarditis |
| 35 | 238 D | epi. carcinoma | 38x15x4 | submucosa | Lung abscesses |
| 36 | 244 D | epi. carcinoma | 32x12x3 | muscle | Bronchitis |
| 37 | 252 S | papilloma | 1x1x0.5 multiple | | |
| 38 | 252 S | epi. carcinoma | 8x8x3 | subsmucosa | Lung abscesses |
| 39 | 252 S | epi. carcinoma | 17x12x3 | submucosa | |
| 40 | 252 S | epi. carcinoma | 40x15x10 | serosa | Bronchitis |
| 41 | 252 S | epi. carcinoma | 16x14x10 | serosa | Bronchiectasis |

D: died S: sacrificed

strong carcinogen for production of adenocarcinoma in stomach. But a long time is necessitated before carcinoma can be recognized.

In order to shorten the induction period and increase induction rate, a high concentration of MNNG was used. For the same reasons. gastric tube feeding was selected in this experiment, aimed to bring higher concentration of MNNG to the target organ. Due to the high death rate before the completion of the experiment and the high percentage of aspiration pneumonia of the dead animals, the gastric tube method was thought to be unpractical unless carried out by an experienced person.

Administration of a saturated salt solution during the experiment was based on the work of Capoferro and Torgersen[12], who reported that hypertonic saline enhanced the uptake of 7,12-dimethyl-benzanthracence. We hoped that the addition of NaCl can also increase the uptake of the MNNG. But this problem is still unknown because no control was set in this experiment.

It is well known that one kind of carcinogen can produce many kinds of tumor, if the target organ or method of application varies. MNNG has been found to have the ability of producing many kinds of tumor in many kinds of animals[9,19,13-23] 1) fibrosarcoma in rat if injected subcutaneously (Sugimura et al 1966)[10] 2) Epidermoid carcinoma on rat stomach if given in 30% ethanol solution (Schoental 1966)[9] 3) and adenocarcinoma of rat glandular stomach given in a concentration of 0.033 mg% for 12 months or 0.083 mg% for months (Sugimura and Fugimura 1967)[13] 4) adenocarcinoma of colon and rectum in rat by rectal infusion method in a concentration of 1.25 mg% for 32 days (Narisawa et al 1971)[22] and skin cancer (fibrosarcoma) in mice through direct application (Takayama et al 1971)[23].

In our experiment, the concentration of MNNG was kept at 1.5 mg% per ml per day. The earliest production of tumor was at 67 days and the earliest production of malignant tumor 169 days after the start of the experiment, respectively. The over-all production rate was 60.0%, but production rate on the group of animals tolerated more than 30 wks was 100%. (Table 3) Thus the induction period markedly shortened and production rate markedly increased if the concentration of MNNG was increased.

**Table 3. Induction Rate of Tumor in 41 Rats**

| Duration (wk) | No. of Animal | Papilloma | Carcinoma | Induction Rate (%) | | |
|---|---|---|---|---|---|---|
| | | | | Papilloma | Carcinoma | Tumor |
| 1–10 | 5 | 1 | 0 | 20.0 | 0 | 20.0 |
| 11–20 | 18 | 8 | 0 | 44.4 | 0 | 44.4 |
| 21–30 | 8 | 2 | 4 | 25.0 | 50.0 | 75.0 |
| 30– | 10 | 2 | 8 | 20.0 | 80.0 | 100.0 |
| Total | 41 | 13 | 12 | 31.7 | 29.3 | 60.0 |

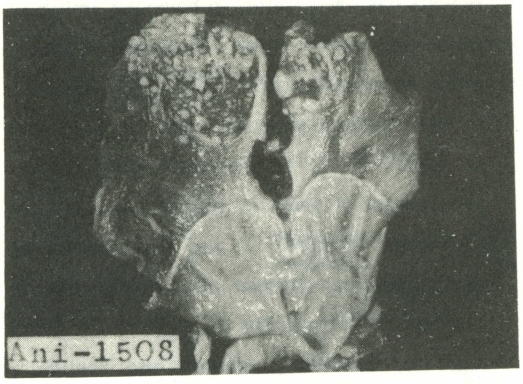



Fig. 1. Multiple papilloma on fundus.

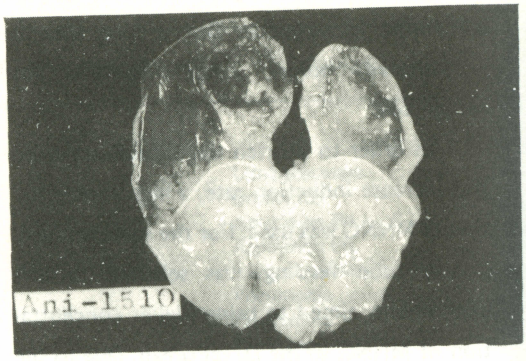



Fig. 2. A protruding carcinoma about 5 mm in diameter at anterior fundus. A few papilloma and hyperkeratosis on posterior fundus and lesser curvature.

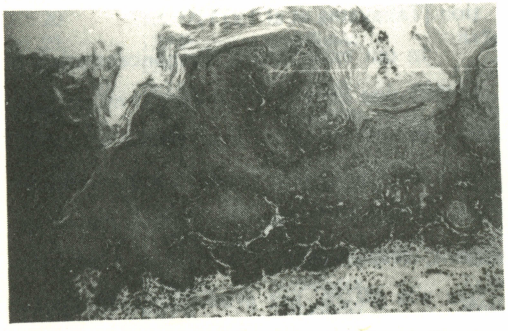


Fig. 3. Wide papillomatous growth. A few dark staining small nests beginning to invade muscularis mucosaris probably represents carcinomatous change of the papilloma.

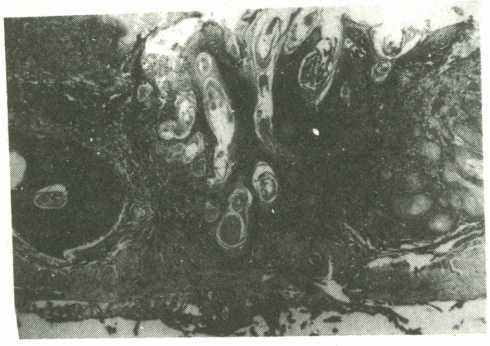


Fig. 4. A small epidermoid carcinoma beginning to penetrate into the inner muscle layer.

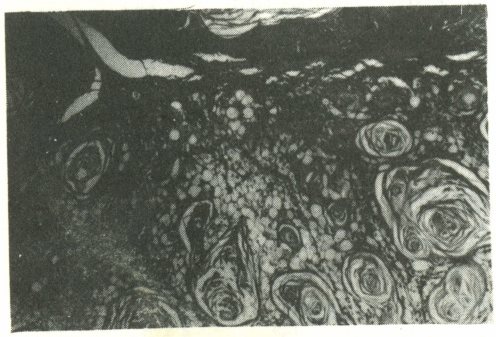


Fig. 6. A carcinoma contains central epithelial pearls but other cells are all large clear cells resembling sebaceous cells.

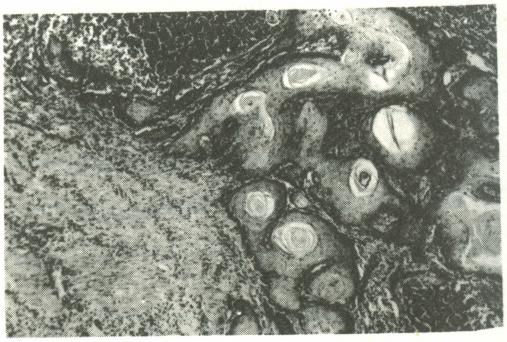


Fig. 5. Higher magnification of an epidermoid carcinoma showing marked tendency toward central keratinization of the nest. To the left is muscle layer of the stomach.

In 1967 Sugimura and Fujimura[13] reported that MNNG administered to rats at the concentration of 83 mg/l in drinking water led to adenocarcinoma of the glandular stomach in 70% of animals within 1 year. In 1970, Bralow et al[15] also obtained the same result. The induction of squamous carcinoma in the forestomach of rat was first reported by Schoental[9] in 1966. Administration of MNNG by stomach tube in 50-100 mg/kg for 1-2 years induced squamous carcinoma. In 1968, Craddock[14] also obtained the same result by intragastric administration of MNNG with the concentration of 20 mg/2 ml. Fujimura et al [16] in 1970 found if the concentration of MNNG was raised up to 167 ug/ml, it would increase the incidence of squamous cell papilloma (42.5%, 295 days).

From the above, we conclude that if the MNNG is given in a low concentration over a longer period in rat, it produces adenocarcinoma in glandular stomach; but if given in higher concentration, the induction period can shorten and the induction rate can increase, but the tumor produced is epidermoid carcinoma.

Why the variation in concentration of one kind of carcinogen on the same kind of animal produces different kinds of tumor is not known. It may be due to 1) formation of a new substance by biochemical synthesis, decomposition or substitution of the carcinogen with the various kinds of extracellular or intracellular substances, 2) alteration of the pathway of MNNG to cell membrane or intracellular microorganeless, or 3) causing a difference in acting site of the carcinogen, thus the mechanism of action. All of these need further study.

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stomach of the rat. J. Natl. Cancer Inst. 55; 1395-1401, 1975.

# N—甲基—N′—硝基—N—亞硝基胍的致癌性研究

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N—甲基—N′—硝基—N—亞硝基胍（MNNG）是個常見的致癌物質，其接觸途徑或濃度的不同，則在不同的部位起不同的癌變化，本實驗乃針對此作研究。

以胃管每天灌服1.5毫克／毫升的MNNG溶液，並每週灌服1毫升之飽和食塩水，共給予15週後停止給致癌物及食塩水，待實驗結果（36週），解剖鏡檢，發現病變大部份爲鱗狀細胞癌且多發生在前胃部。若以超過實驗30週後之老鼠來觀察其致癌的情形，其致癌率高達100%，由此看來MNNG毫無疑問的是一種不容忽視的強致癌物。

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